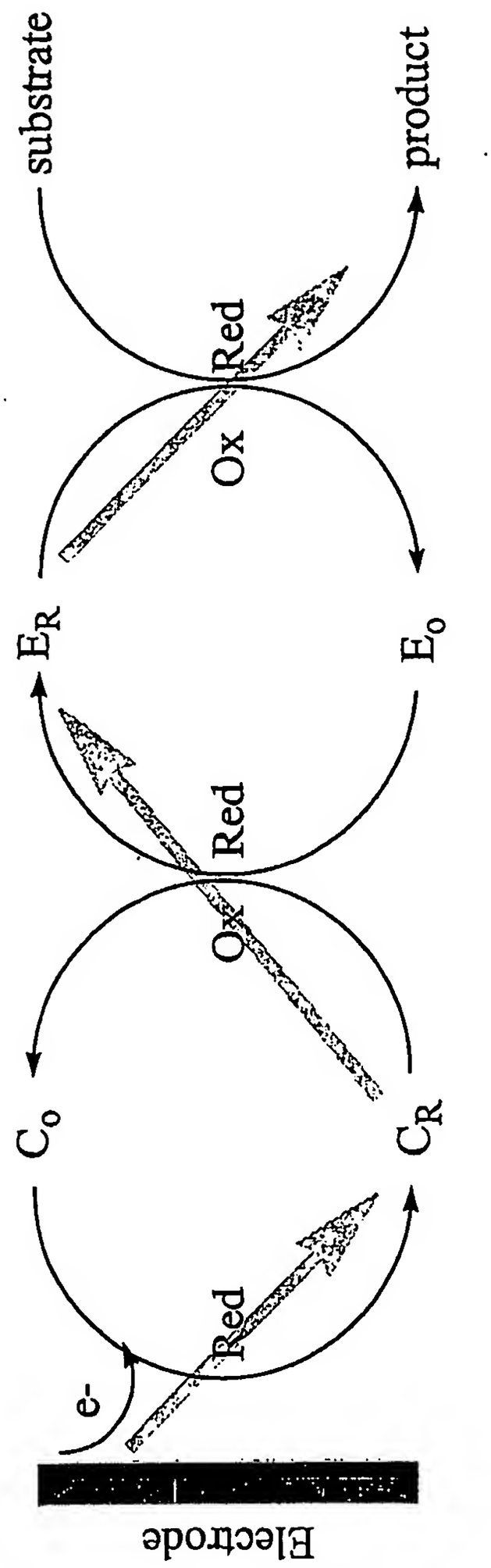
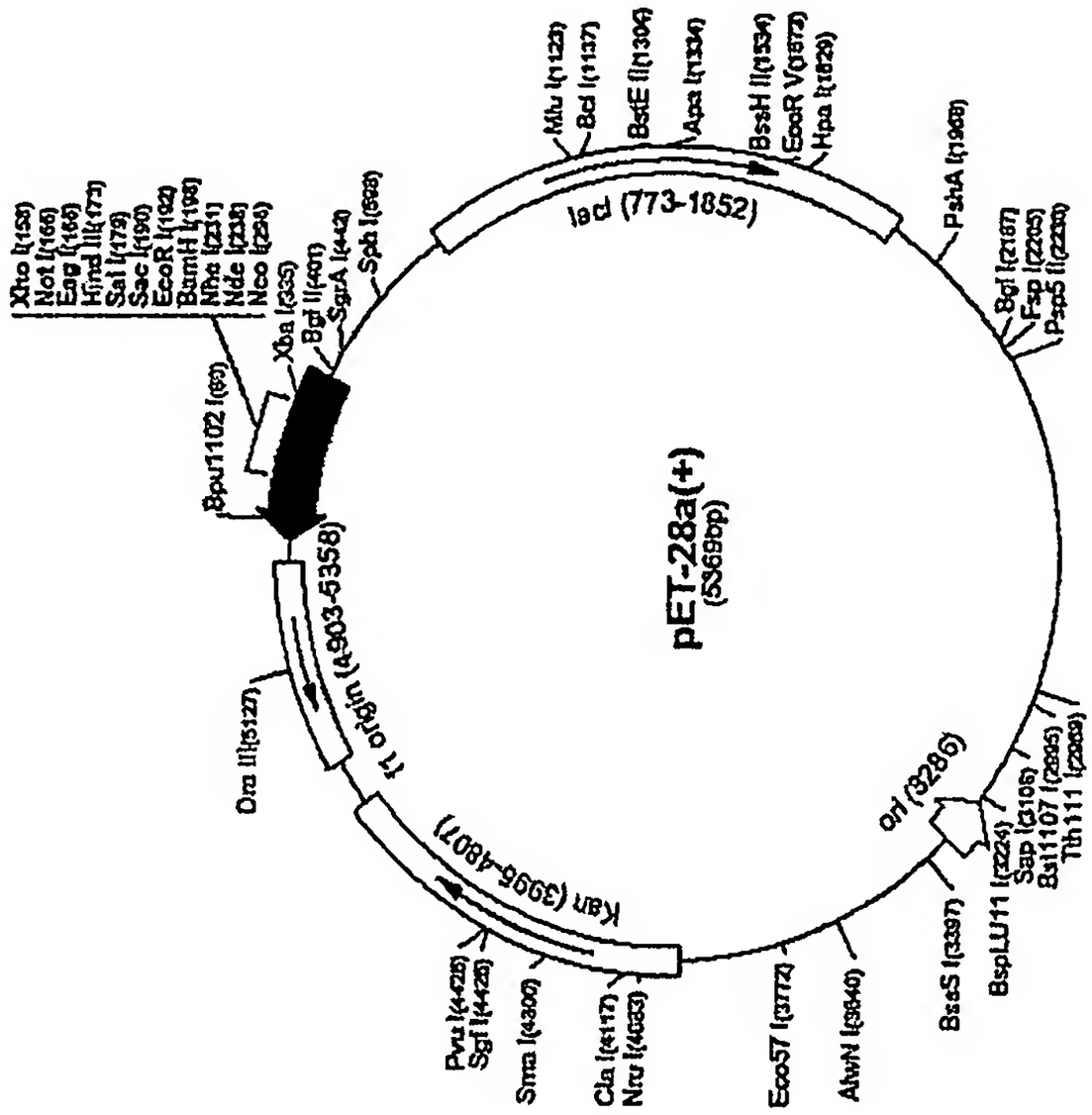


Figure 1

Figure 2


pET-28a(+)
(5368bp)
lacI (773-1852)
T7 promoter (4903-5358)
Kan (3996-4807)
ori (3286)
PshA (1968)
BsaS I(2397)
AlwN I(3840)
Eco57 I(3772)
Cla I(4117)
Nru I(4033)
Sma I(4306)
Pvu I(4426)
Sgf I(4428)
Ora II(5127)
Bpu1102 I(65)
Xho I(150)
Not I(166)
Eag I(166)
Hind III(173)
Sal I(179)
Sac I(190)
EcoR I(196)
BamH I(198)
Nhe I(231)
Nde I(235)
Nco I(254)
Xba I(335)
Bgl II(401)
SgrA I(442)
Sph I(668)
Mlu I(1123)
Bcl I(1137)
BstE II(1304)
Apa I(1334)
BssH II(1536)
EcoR V(1672)
Hpa I(1829)
Bgl I(2187)
Fse I(2205)
Pac I(2220)
BspLU11 I(2224)
Sap I(2106)
Bsi1107 I(2895)
Tth111 I(2909)

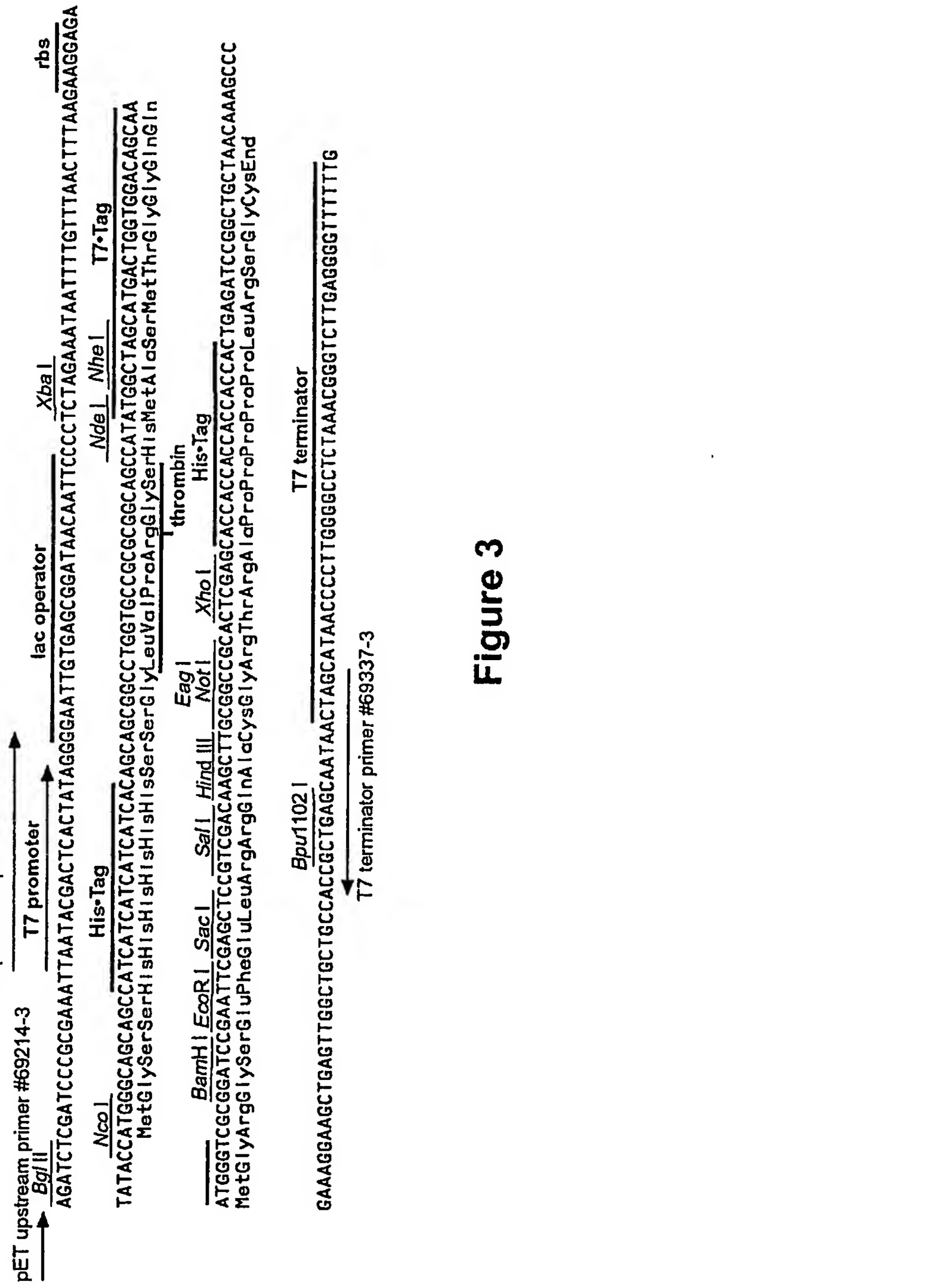

T7 promoter primer #69348-3
T7 promoter
lac operator
rbs
TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAA
His-Tag
CATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGCTAGCATGACTGGTGGA
-HisHisHisHisHisHisSerSerGlyLeuValProArgGlySerHisMetAlaSerMetThrGlyGly
thrombin
EcoR I Sac I Sal I Hind III Not I Xho I
ATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACTGAGATCCGGCTGC
PheGluLeuArgArgGlnAlaCysGlyArgThrArgAlaProProProProProLeuArgSerGlyCys
Bpu1102 I
GCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTG
T7 terminator primer #69337-3
T7 terminator
pET upstream primer #69214-3
Bgl II
AGATCTCGATCCCGCGAAATT
Nco I
TATACCATGGGCAGCAGCCAT
MetGlySerSerHis
BamH I EcoR I
ATGGGTCGCGGATCCGAATTC
MetGlyArgGlySerGluPhe
GAAAGGAAGCTGAGTTGGCTG

Figure 3

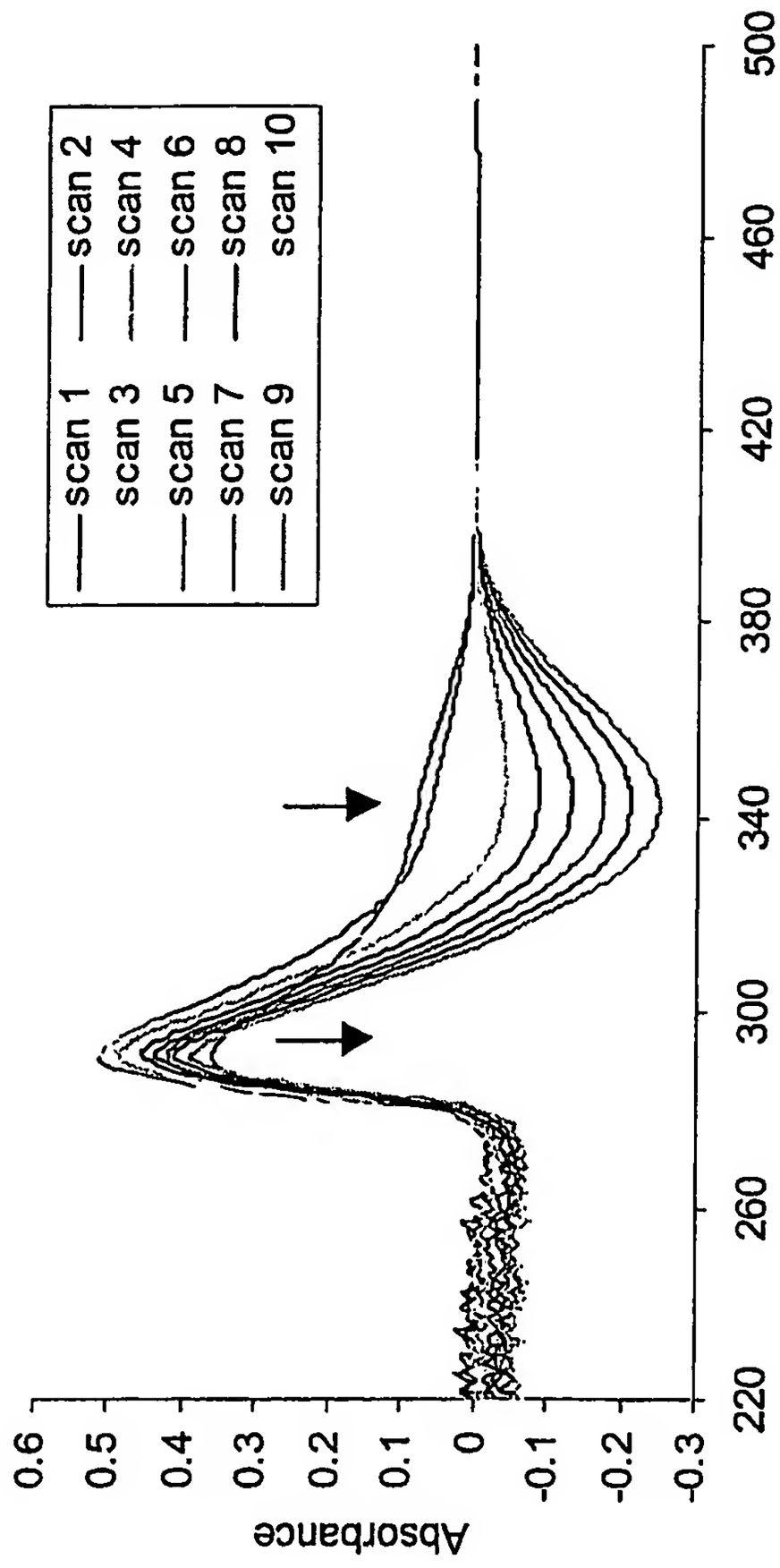

scan 1
scan 2
scan 3
scan 4
scan 5
scan 6
scan 7
scan 8
scan 9
scan 10
Absorbance
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
220
260
300
340
380
420
460
500
Wavelength nm

Figure 4

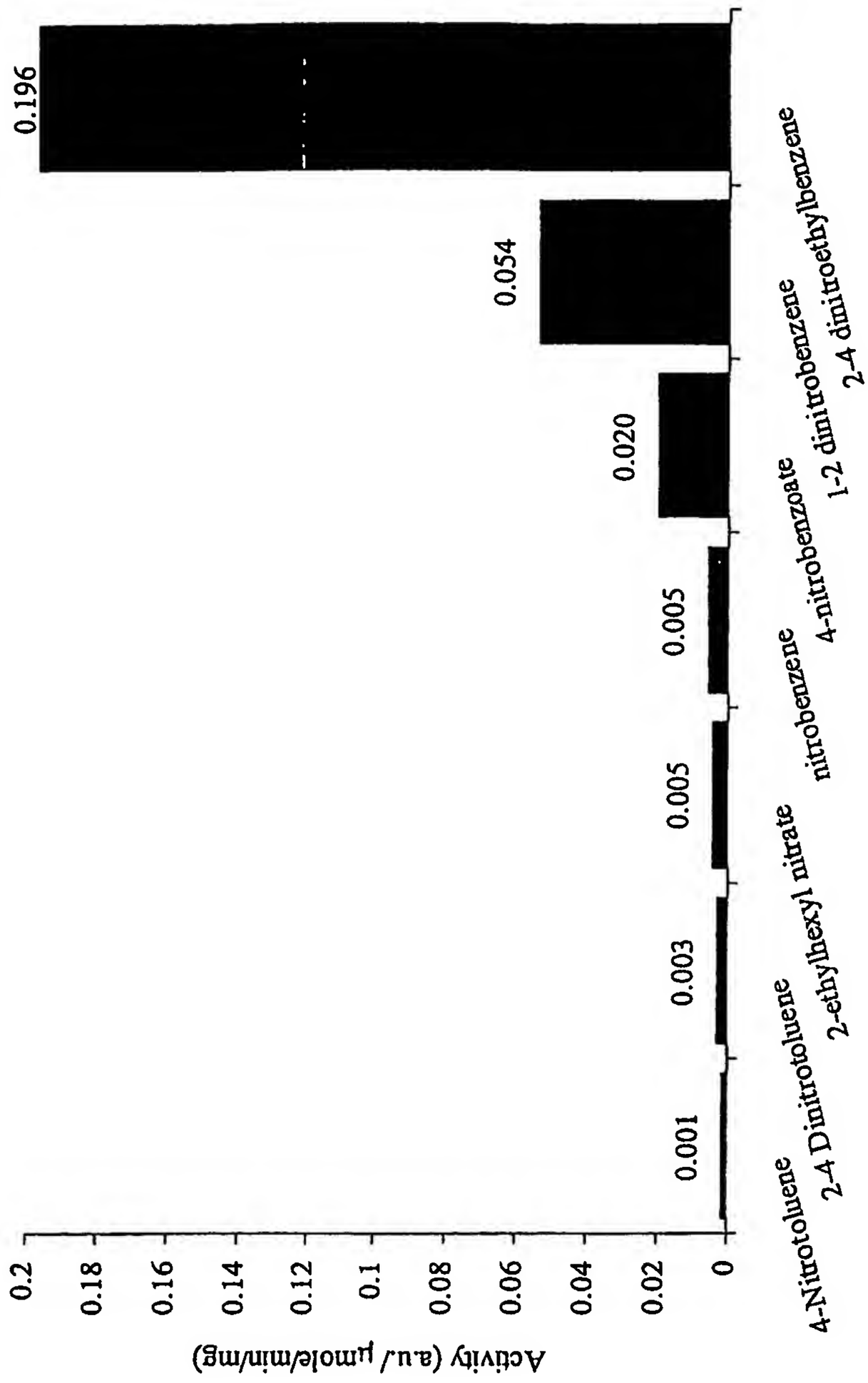

Activity (a.u./ µmole/min/mg)
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0.196
0.054
0.020
0.005
0.005
0.003
0.001
4-Nitrotoluene
2-4 Dinitrotoluene
2-ethylhexyl nitrate
nitrobenzene
4-nitrobenzoate
1-2 dinitrobenzene
2-4 dinitroethylbenzene

Figure 5

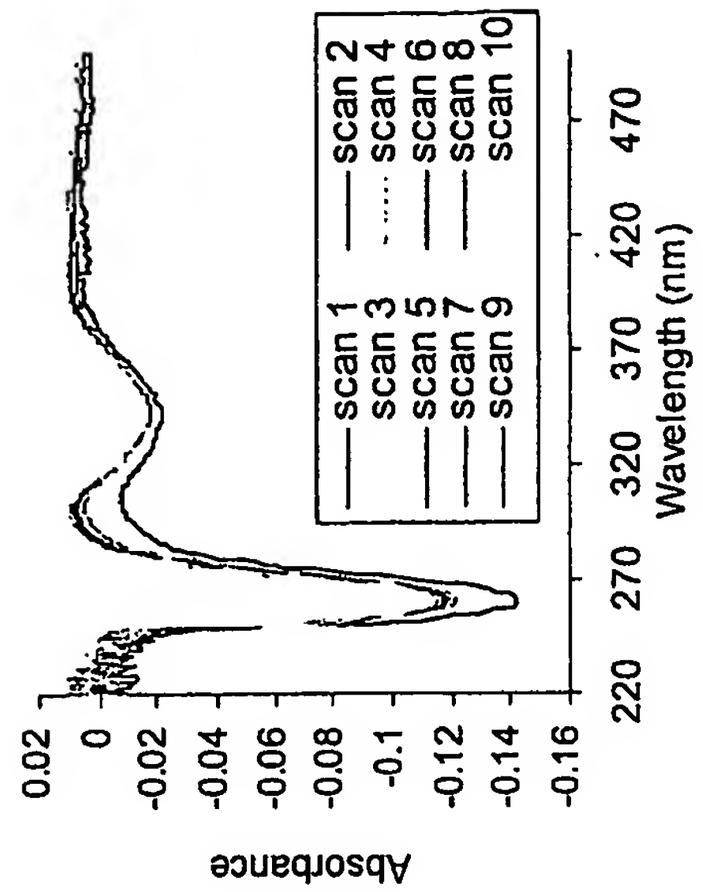


Figure 6B

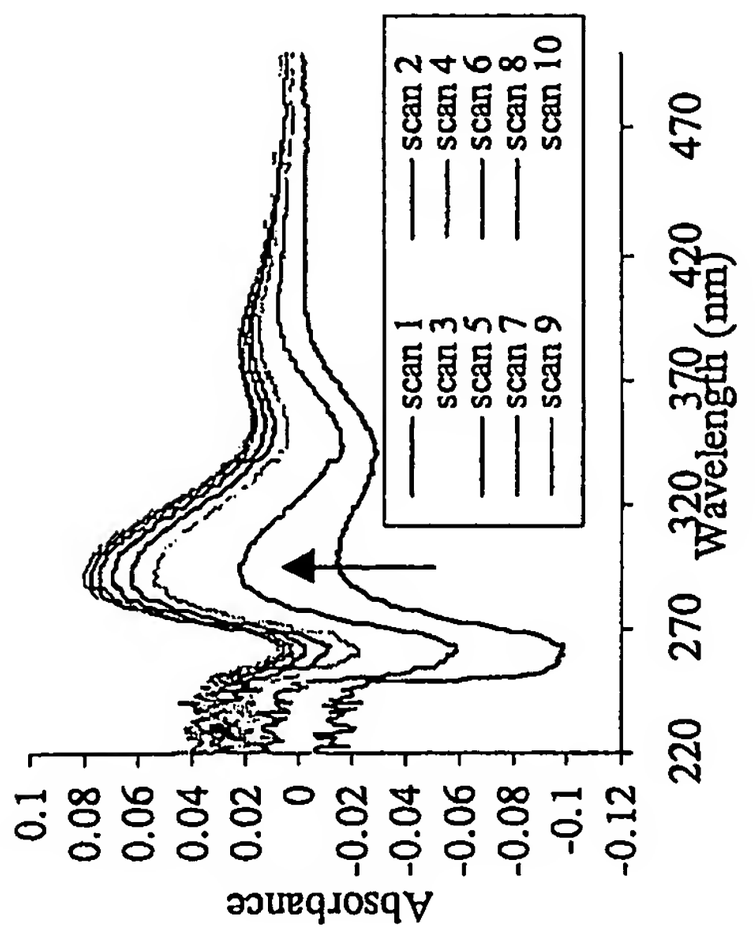


Figure 6A

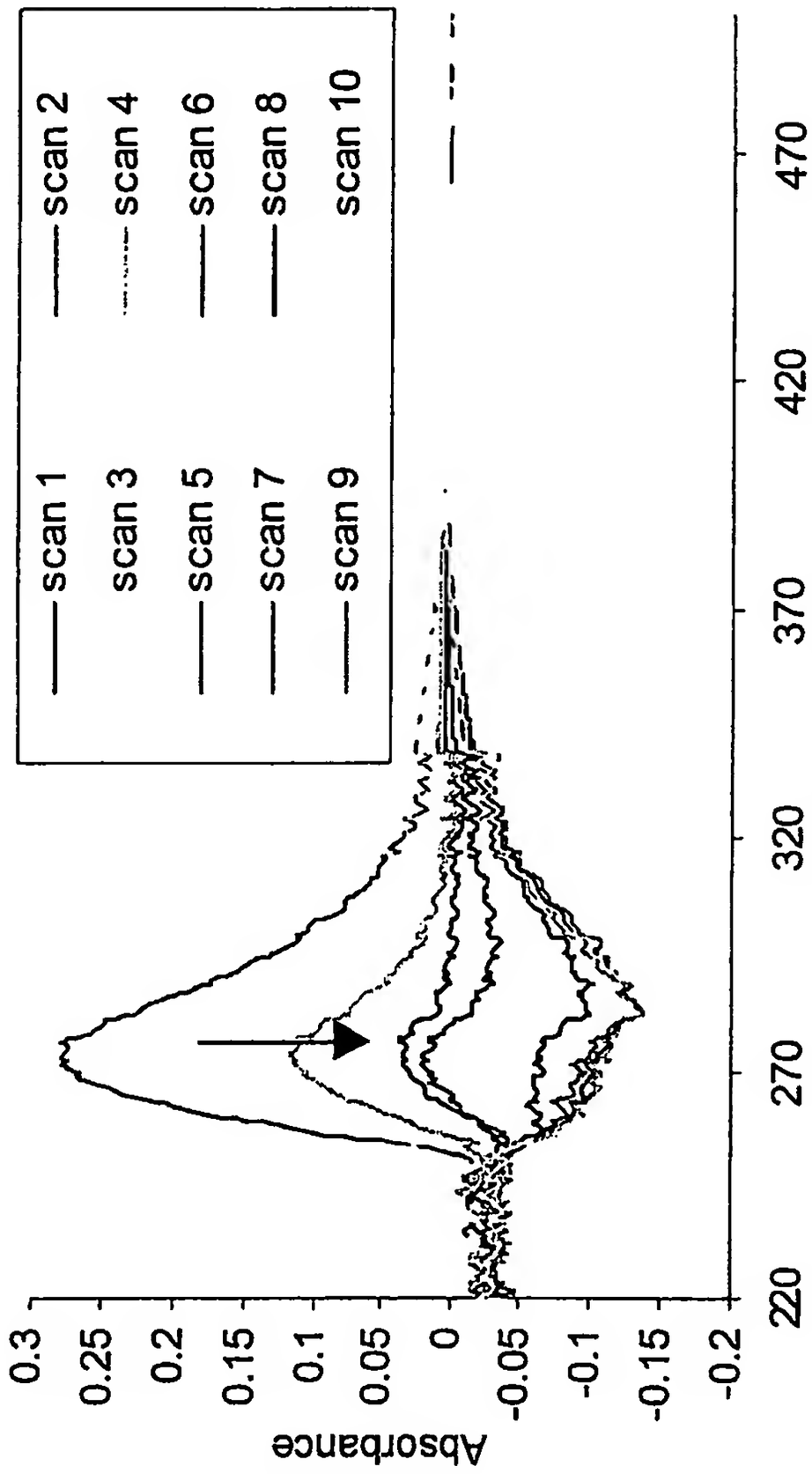

scan 1
scan 2
scan 3
scan 4
scan 5
scan 6
scan 7
scan 8
scan 9
scan 10
470
420
370
320
270
220
Wavelength (nm)
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
Absorbance

Figure 7

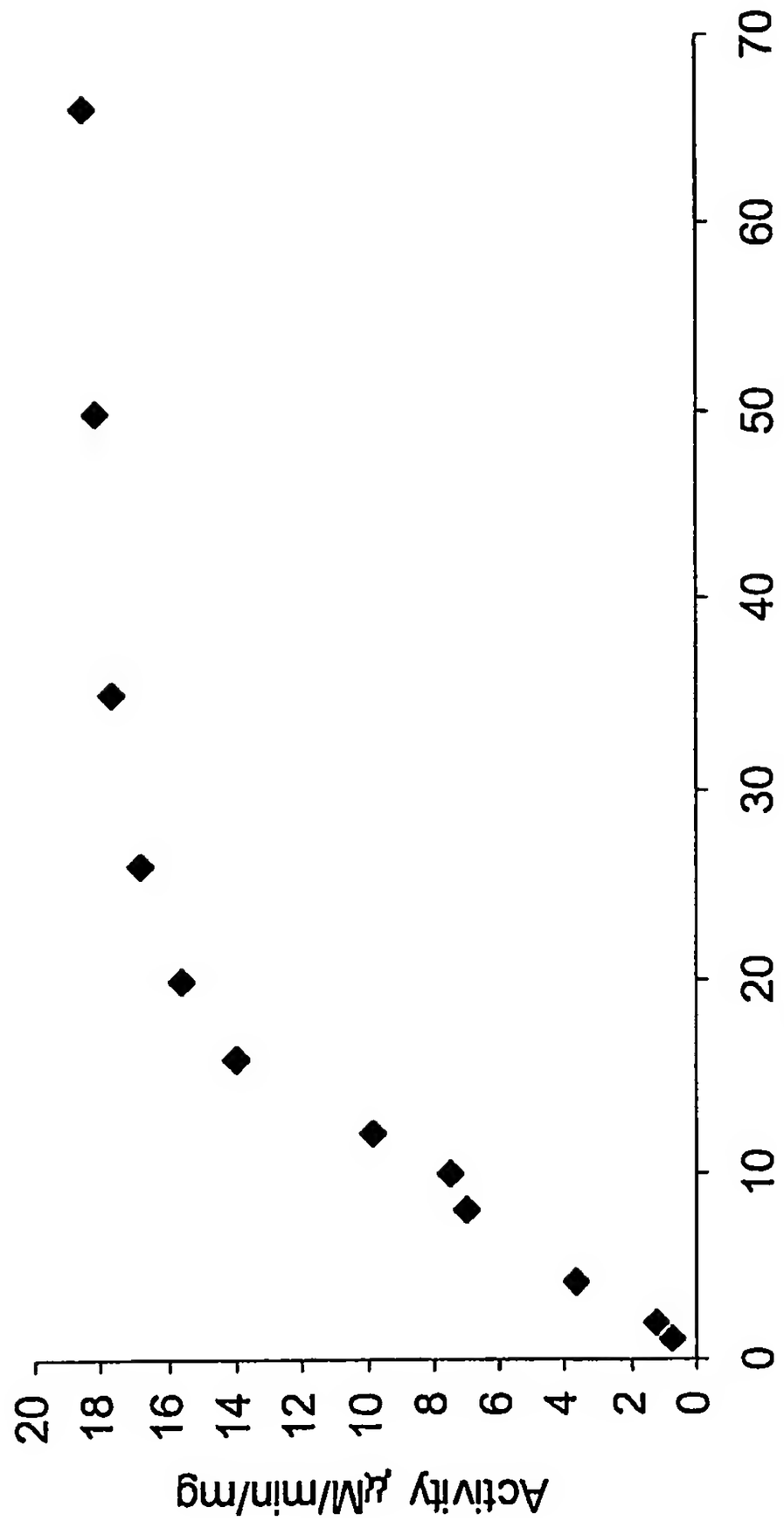

Activity µM/min/mg
0
2
4
6
8
10
12
14
16
18
20
µM substrate (2,4 dinitroethylbenzene)
0
10
20
30
40
50
60
70

Figure 8

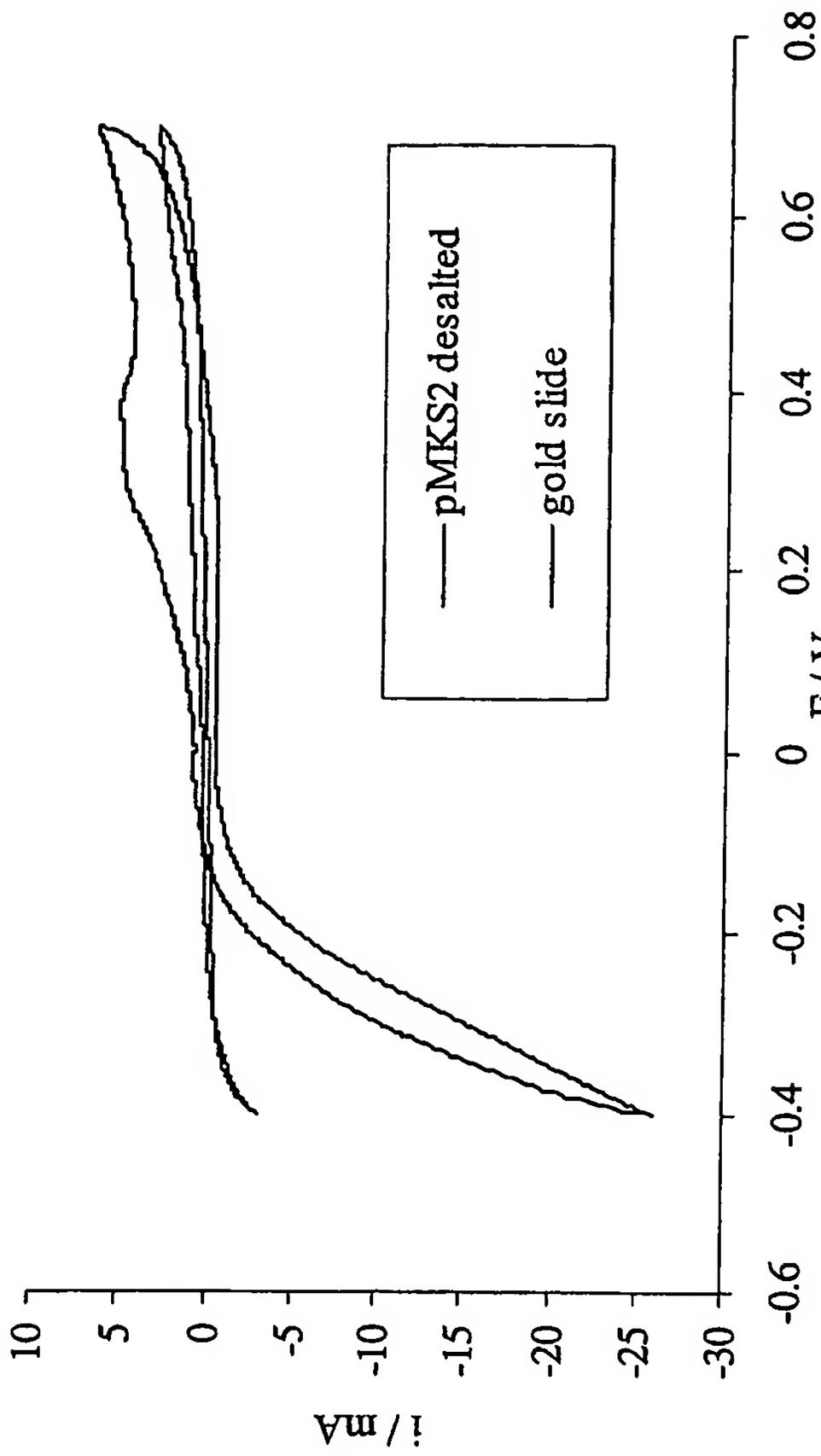

pMKS2 desalted
gold slide
i / mA
10
5
0
-5
-10
-15
-20
-25
-30
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
E / V

Figure 9

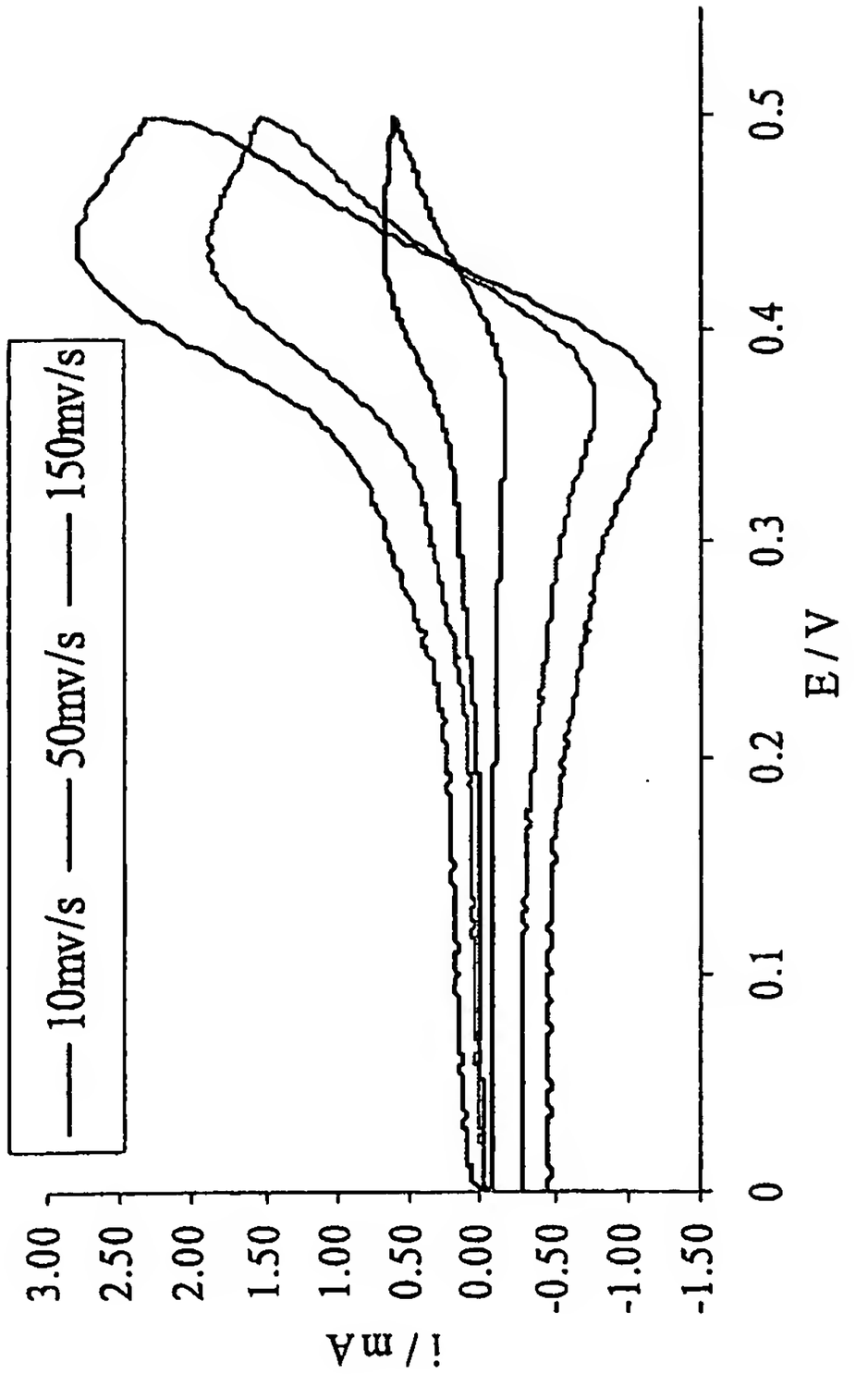

10mv/s
50mv/s
150mv/s
E / V
0
0.1
0.2
0.3
0.4
0.5
i / mA
3.00
2.50
2.00
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50

Figure 10

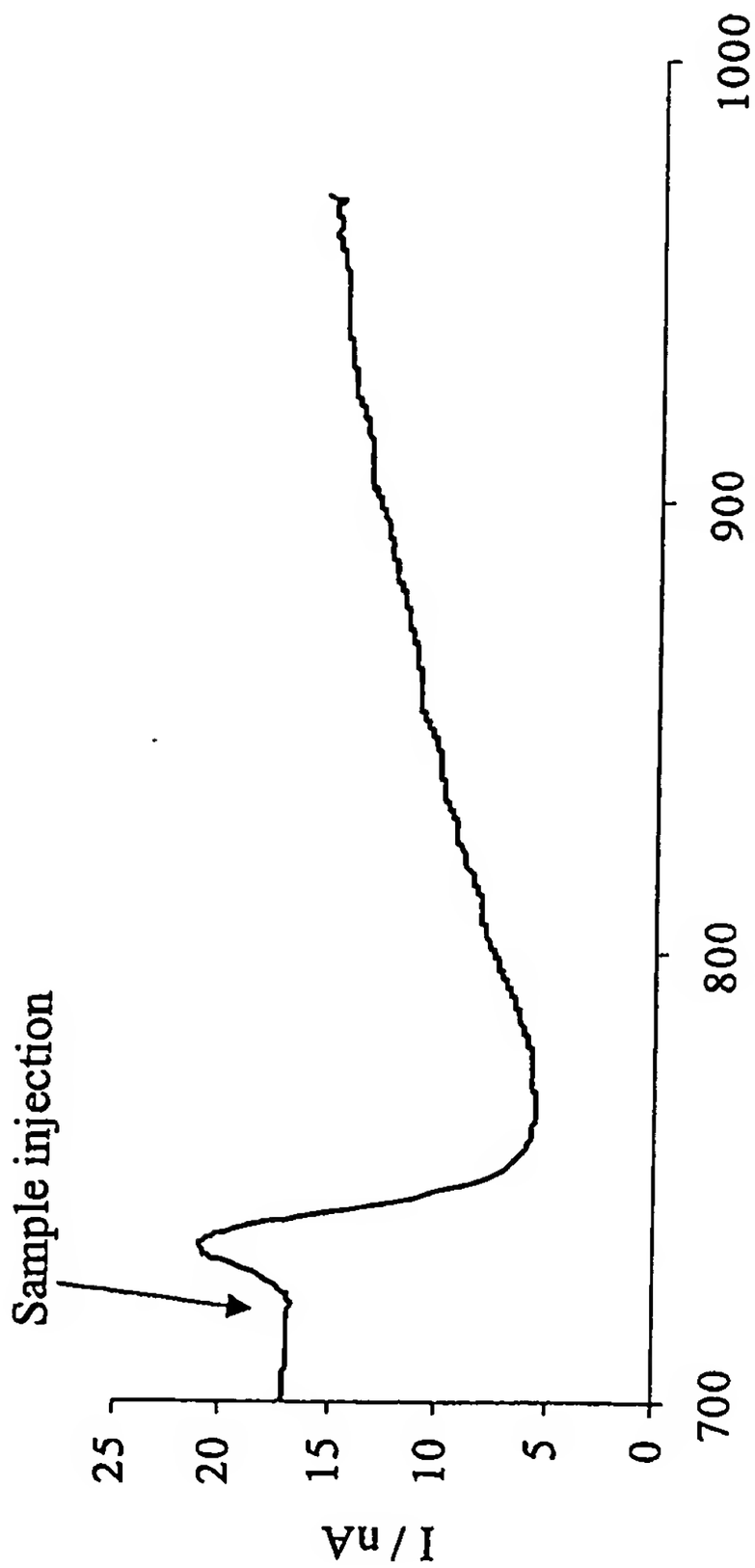


Figure 11

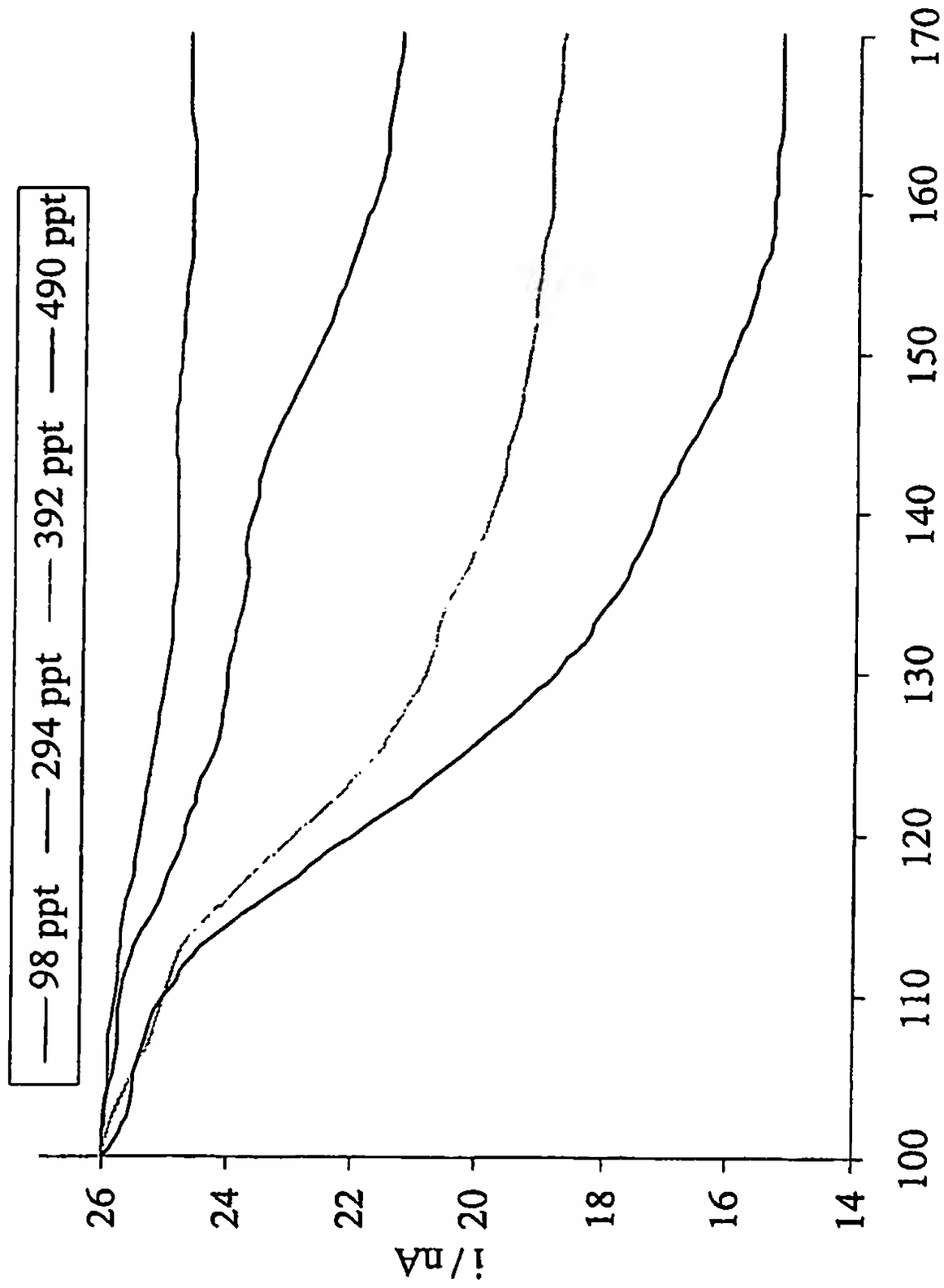

98 ppt
294 ppt
392 ppt
490 ppt
i / nA
t / s
26
24
22
20
18
16
14
110
120
130
140
150
160
100

Figure 12A

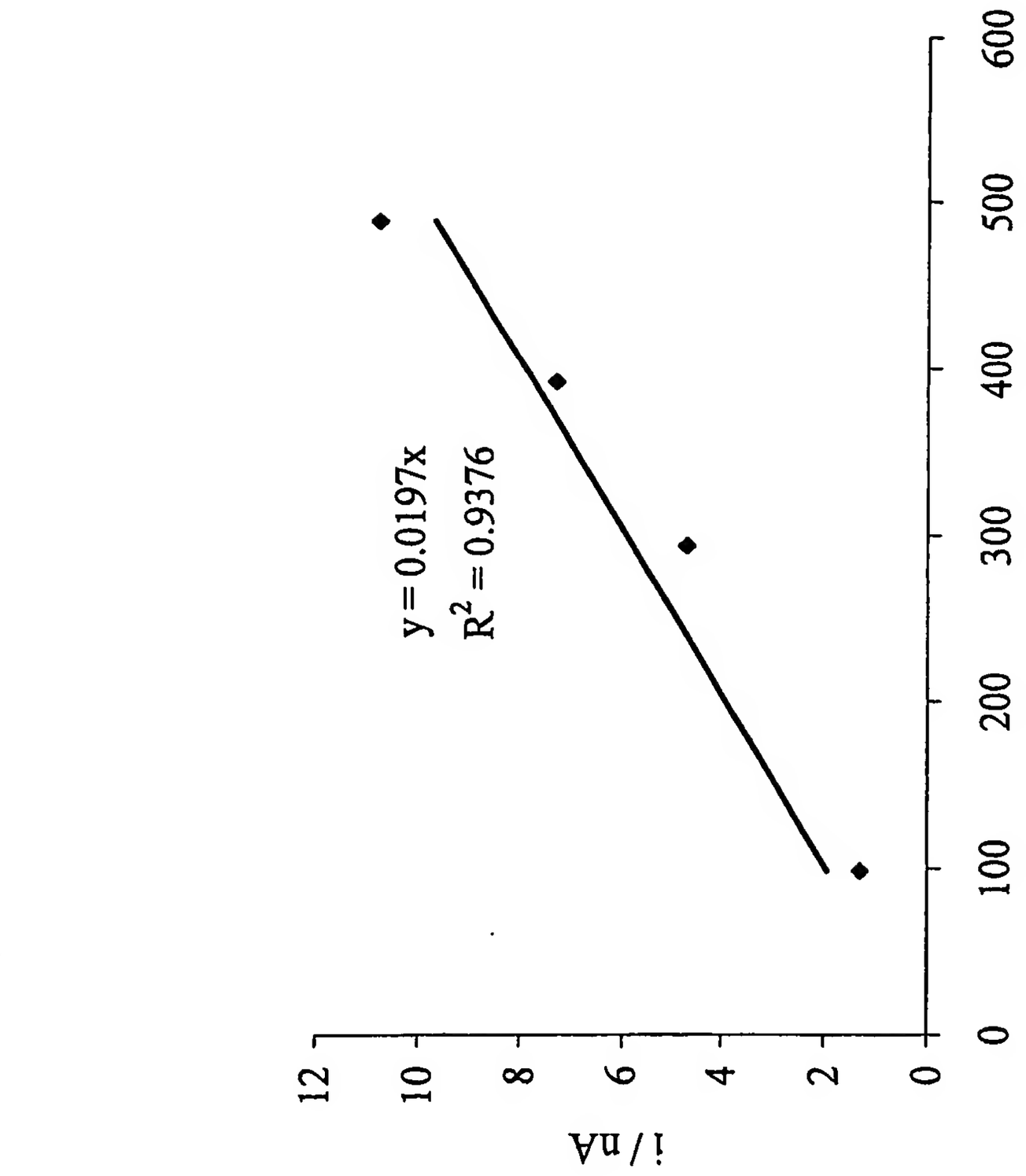

y = 0.0197x
R² = 0.9376
i / nA
ppt(mass)
0
2
4
6
8
10
12
0
100
200
300
400
500
600

Figure 12B